The management of residual disease is a central problem in breast and other solid tumors. Despite efforts to maximize dose intensity, relapse remains a critical and generally fatal problem in high risk breast cancer patients. Chemotherapeutic strategies are necessarily limited by various toxicities, and of limited efficacy against nonproliferating tumor cells. Additional modalities, which will achieve further cytoreduction are needed. A variety of investigators have suggested the use of gene transfer techniques to augment immunogenicity of cancer cells, and provoke an immune tumor-directed response. Many of these strategies involve *ex vivo* manipulation of tumor cells, are technically difficult to implement, and do not target systemic tumor deposits.

Although various different trials of monoclonal antibodies, antibody based conjugates and/or radioantibody have been performed, with limited success, results of these trials have highlighted obstacles to successful antibody therapy of human malignancy. Antibody opsonization generally does not result in direct cytotoxicity, due to poor fixation of complement and/or poor enlistment of antibody dependent cytotoxicity (ADCC). Strategies based on direct antibody-based killing (e.g. antibody-toxin conjugates such as antibody-ricin, or radiolabeled antibody strategies, e.g. ^{131}I-Ab) require delivery to all tumor cells and are hampered by limited vascular permeability to proteins of 150kd or greater (mw of IgG) and extravascular diffusion ability. Elevated interstitial pressures within tumor masses due to absent/poorly organized lymphatics further impede delivery. Antibody (Ab) and cytokine activation of effector cells may be more effective than Ab alone.

Stimulation of an antitumor immune response is a stepwise process requiring the accumulation and activation of immune effector cells in the vicinity of tumor cells. Monocytes and lymphocytes initially interact with adhesion molecules on endothelial cells, followed by migration of immune effector cells in response to chemotactic gradients in tissues. Effector cells in the tumor vicinity are then available for activation and subsequent stimulation of an antitumor immune response. Chemokines are low molecular weight proteins that act as potent chemoattractants, and are involved in migration of inflammatory cells. They are divided according to the configuration of the first cysteine residues at the amino terminus of the protein. Different subfamilies of chemokines have been shown to attract different classes of inflammatory cells. C-C chemokines predominantly attract monocytes and lymphocytes, while C-X-C chemokines attract neutrophils in addition to lymphocytes. RANTES is a member of the C-C chemokine family and is a potent chemoattractant of T cells, NK cells, monocytes, eosinophils, basophils, and dendritic cells.

RANTES, present at high concentrations (1μM), has also been shown to stimulate T cell activation and proliferation. RANTES-mediated T cell activation can also lead to the generation of an antitumor immune response and tumor rejection as shown in gene transfer studies performed in murine syngeneic *in vivo* EL4 lymphoma and MCA-205 tumor models. Therefore, direct delivery of RANTES to tumor deposits may assist in recruitment and/or the molecule may be used as a modulator for cancer immunotherapy.

T-cell activation and proliferation requires two signals from antigen-presenting cells (APCs). The first signal is antigen specific and mediated by recognition of antigenic peptides presented in the context of MHC-I or II by the T-cell receptor (TCR). A second or "costimulatory" signal can be provided *via* binding of a costimulatory ligand of the B7 family on the APC to the CD28 counterreceptor present on T-cells. The B7 family includes several Ig-like molecules including B7.1 and B7.2. Provision of signal 1 without signal 2 may lead to a state of immune tolerance. B7.1 gene transfer into nonimmunogenic tumor cells has been shown to elicit a T-cell-mediated immune response not only against transfected (B7+) but also against parental nontransfected tumor cells. Since T-cell activation requires both B7.1 activation and TCR engagement, only cells with TCRs which recognize antigenic determinants on tumor cells should be activated.

Chemical conjugation of antibody to cytokines instead of fusion has resulted in decreased T-cell activation by the conjugate although effects on vascular permeability are preserved. In contrast, recent studies using an anti-tumor antibody-IL-2 fusion protein suggest retention of both antibody specificity and cytokine function in the fusion molecule.

B7.1 gene transfer is not always a realistic option for treating cancer in a mammal. B7.1 gene transfer requires either *ex vivo* manipulation of tumor cells which is technically difficult, or *in vivo* delivery *via* gene therapy vectors which would not specifically target systemic tumor deposits. An effective method would not rely on absolute kill of all tumor cells by antibody/conjugate nor upon delivery to all tumor cells to elicit a response. The present invention overcomes the significant problems with biodistribution and delivery associated with prior methods.

The objection to the drawings is respectfully traversed in view of the submission of formal drawings appended to the accompanying Letter to Official Draftsman.

The objection to the specification is respectfully traversed in view of the above amendments.

The rejection of claims 1-10 and 25 under 35 U.S.C. § 112 (2nd para.) is respectfully traversed in view of the above amendments. As to the "chemokine or active fragment" limitation in the claims, it is noted on page 3, lines 11-19 of the present application that chemokines are said to be low molecular weight proteins that act as potent chemoattractants and are involved in the migration of inflammatory cells. Chemokines are divided into different families according to the configuration of the first cysteine residues at the N terminus of the protein with different families of chemokines attracting different classes of inflammatory cells. In particular, C-C chemokines predominantly attract monocytes and lymphocytes, while C-X-C chemokines attract neutrophils in addition to lymphocytes. Examples of chemokines which are useful in carrying out the present invention are set forth on page 14, lines 27-31. From this information in the specification, one of ordinary skill in the art would recognize that an active fragment of a chemokine would be portions of chemokines capable of functioning as chemoattractants and involved in migration of inflammatory cells. Accordingly, it is submitted that the "chemokine or active fragment" claim limitation fully satisfies 35 U.S.C. § 112 (2nd para.).

The rejection of claims 1-10 and 25 under 35 U.S.C. § 112 (1st para.) is respectfully traversed. Applicants submit that from the disclosure of the present application, one of ordinary skill in the art would be fully able to practice the present invention with binding domains other than antibodies and fragments thereof. Ligands which bind to tumor cell associated antigens are well known to those of ordinary skill in the art. For example, U.S. Patent No. 5,514,554 to Bacus ("Bacus") teaches that the her2/neu oncogene is not only recognized by monoclonal antibodies but also by ligands, such as gp39 and NDF. See col. 7, lines 11-15 of Bacus. In view of this knowledge by those skilled in the art, it is inappropriate to limit the claimed invention to antibodies and fragments thereof. Therefore, the rejection under 35 U.S.C. § 112 (1st para.) should be withdrawn.

The rejection of claims 1-2, 5-6, 8, 10, and 25 under 35 U.S.C. § 102(a) as anticipated by Hölzer, et. al., "A Fusion Protein of IL-8 and a FAB Antibody Fragment Binds to IL-8 Receptors and Induces Neutrophil Activation," Cytokine 8(3): 214-22 (1996)("Hölzer article") is respectfully traversed.

The Hölzer article discloses a fusion protein which combines an Fab fragment of a monoclonal antibody which is directed to the human epidermal growth factor receptor and the biologically active N-terminally truncated 2-72 amino acid form of the human chemokine IL-8. The fusion protein is formed by removing the N-terminal serine of IL-8 and

adding 3 amino acids (i.e. Ala-Met-Gly) between IL-8 and the heavy chain of the Fab fragment from the monoclonal antibody. Although the N-terminus of IL-8 is the portion of this chemokine which interacts with the IL-8 receptor, it was found that N-terminal fusion of IL-8 to the carboxyl terminus of the Fab fragment did still bind to the IL-8 receptor, albeit at a reduced level.

The claimed chimeric molecule is readily distinguishable from the fusion protein of the Hölzer article. In particular, the claims of the present application call for "a chemokine or active fragment thereof, which is coupled to the N terminus of the binding domain". Support for this feature is found on page 45, lines 2-7 and page 46, lines 24-28 of the specification. By contrast, the Hölzer article's fusion protein binds the N-terminus of IL-8 (i.e. the chemokine) to the carboxy terminus of the Fab fragment of the monoclonal antibody (i.e. the binding domain). The Hölzer article indicates that this construction reduces the level of binding between IL-8 and the IL-8 receptor. To the extent the Fab fragment's interference with such binding becomes too great, the Hölzer article suggests that a linker can be added by means of the restriction site between the Fab fragment and the IL-8. See page 218, column 2, end of last full paragraph of the Hölzer article. The presence of such a linker-containing fusion protein is said to impart "sufficient mobility to expose the ELR motif and to allow receptor binding and signal transduction". By contrast, the chimeric molecule of the present invention has little effect on the binding domain's ability to couple to its antigen. Nowhere does the Hölzer article suggest coupling the chemokine to the N terminus of the binding domain. Therefore, the rejection based on this reference should be withdrawn.

The rejection of claims 1-2, 5-8, 10, and 25 under 35 U.S.C. § 102(e) as anticipated by U.S. Patent No. 5,824,782 to Hölzer et. al., ("Hölzer patent") is respectfully traversed. The Hölzer patent is substantially the same as the Hölzer article. Therefore, the Hölzer patent is distinguishable from the claimed invention for substantially the same reasons as the Hölzer article, as set forth *supra*.

The rejection of claim 9 under 35 U.S.C. § 103 for obviousness over the Hölzer patent in view of Bacus is respectfully traversed, because Bacus, which is cited as teaching monoclonal antibodies to her2/neu, does not overcome the above-noted deficiencies of the Hölzer patent.

The rejection of claims 1-4 under 35 U.S.C. § 103 for obviousness over the Hölzer patent in view of Huston, et. al., "Protein Engineering of Single-Chain Fv Analogs and Fusion Proteins," Meth. Enzymol. 203: 46-88 (1991)("Huston"), which is cited as

teaching linking to a heavy chain, is respectfully traversed, because Huston does not overcome the above-noted deficiencies of the Hölzer patent.

In view of all the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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